Study Guide 6
Enzyme Kinetics

Multiple Choice Questions

1. How do enzymes (catalyst) increase the rate of a chemical reaction?

   A) Make ΔG more negative
   B) Make ΔG more positive
   C) Lower energy to transition state
   D) Raise energy to transition state
   E) Physically beat the substrate with a bat until it becomes product

2. Which model best describes how enzymes interact with their substrate?

   A) Lock and Key
   B) Induced Fit
   C) Lock and Key and Induced fit are equally good
   D) Model T
   E) The enzyme-ermine model

3. Consider the nerdy enzyme "Daeneryase" that catalyzes the following reaction

   Egg ←→ Dragon

   Would you expect the reaction to exhibit 1st order kinetics?

   A) Yes. There is one product and one reactant
   B) It would depend on the substrate concentration
   C) No, it would be 2nd order
   D) I would like to purchase some of this enzyme
   E) No, it would be zero order

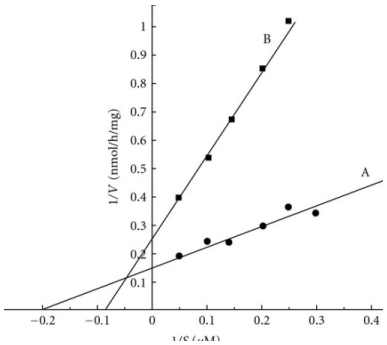
4. Are K_m and V_{max} true constants?

   A) Yes, obviously
   B) No, they vary with experimental conditions
   C) K_m is not, V_{max} is
   D) K_m is, V_{max} is not
   E) There is only 1 constant, my love of biochemistry

5. Enzyme A has a K_m of 0.1mM for glucose and a K_m of 5mM for galactose, which is the preferred substrate?

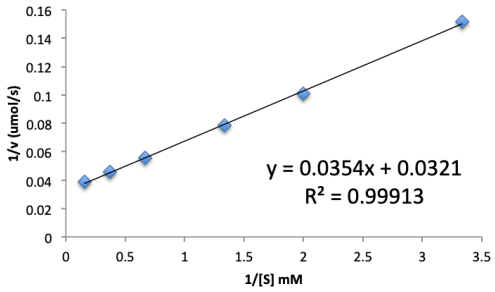
   A) Galactose
   B) Glucose
   C) Sucrose
   D) I like sugar
   E) Equally preferred

6. Two enzymes catalyze a similar reaction, and bind the same substrate. Which has the higher affinity?



A) Enzyme A
B) Enzyme B
C) They are the same
D) You need more info
E) What's an enzyme again?

7. What is the K_m and V_{max} for this enzyme?



A) K_m 0.035, V_{max} 0.032
B) K_m 0.032, V_{max} 0.035
C) K_m 1.1 , V_{max} 31.15
D) K_m 31.15, V_{max} 1.1

8. A competitive inhibitor competes <u>for the same site as the substrate</u>. What effect would you expect it to have on the enzyme?

A) Raise V_{max}
B) Lower V_{max}
C) Raise K_M
D) Lower K_M
E) K_M and V_{max} are affected

9. If an inhibitor bound to a separate site than the active site, and did not affect E + S, what would you expect?

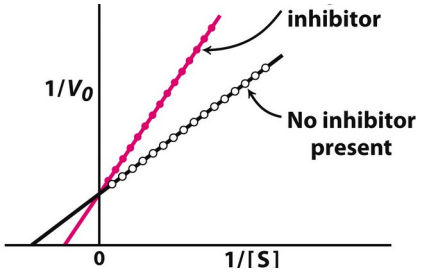
A) K_M would decrease
B) K_M would increase
C) K_M would stay the same
D) V_{max} would stay the same
E) V_{max} and Km would be affected

10. If an inhibitor bound to a separate site than the active site, and affected E + S binding and bound to ES what would you expect?

A) K_M would increase
B) K_M would stay the same
C) V_{max} would stay the same
D) V_{max} and Km would be affected

11. What type of inhibitor is this?

A) Competitive
B) Magical
C) Non-competitive
D) Mixed
E) Uncompetitive



Written and short answer questions

1. Provide an explanation of what K_M and V_{max} are, and also provide a mathematical definition.

2. Under what conditions is K_M an approximation of K_D?

3. What step in Michaelis-Menten kinetics determines the overall rate of the reaction?

4. In the derivation of MM kinetics, several assumptions were made. For each of the assumptions below (i) explain what the assumption means; (ii) how it was useful in the derivation of the MM equation

   a) Only initial rates are measured
   b) Product formation is rate limiting
   c) Steady State Assumption

5. Explain why $k_{cat} = k_2$ in MM kinetics

6. A research group at Fresno State has discovered an enzyme that converts all students that hate biochemistry into students that love biochemistry. This reaction can be simplified into the following enzyme catalyzed equilibrium:

$$\text{HATE-bchem} \quad \leftarrow\rightarrow \quad \text{LOVE-bchem}$$

   a) Using a $[E_t] = 4$ nM, they determine the V_{max} is 1.6 uM s^{-1}. Based on this experiment what is the k_{cat} for this new enzyme?

   b) Using a $[E_t] = 1$ nM and [HATE-bchem] = 30 uM, the researchers determine that V = 300 nM s^{-1}. What is the K_m for HATE-bchem?

   c) Further research shows that the enzyme used in the previous experiments was actually contaminated with a inhibitor called O-CHEM. The researchers remove this horrible inhibitor, and repeat the experiments. They determine that the Km is 15 uM and the V_{max} is 4.8 uM s^{-1}. Based on this information what type of inhibitor was it?

7. Carbonic anhydrate has one of the highest turnover numbers known. It catalyzes the reversible hydration of CO_2

$$H_2O + CO_2 \quad \leftarrow\rightarrow \quad H_2CO_3$$

   If 10 ug of pure carbonic anhydrase catalyzes the hydration of CO_2 in 1 min at 37° C at V_{max}, what is the k_{cat} of carbonic anhydrase (units min^{-1})

8. Measurement of the rate constants for a simple enzymatic reaction obeying Michaelis-Menten kinetics gave the following results:
   $k_1 = 2 \times 10^8\ M^{-1}\ sec^{-1}$
   $k_{-1} = 1 \times 10^3\ sec^{-1}$
   $k_2 = 5 \times 10^3\ sec^{-1}$

   a) What is the dissociation constant (KD) for the enzyme-substrate complex?
   b) What is the K_M?
   c) What is the k_{cat}?
   d) What is the catalytic efficiency of this enzyme? (k_{cat}/K_M)
   e) Does this enzyme approach kinetic perfection?
   f) If a kinetic measurement was made using 2 nanomoles of enzyme per ml and saturating substrate, what would the V_{max} equal?
   g) Again, using 2 nanomoles of enzyme per ml of reaction mixture, what concentration of substrate would give $v = 0.75\ V_{max}$?

9. For each of the following types of inhibitor indicate (i) What the inhibitor binds (ii) the effect on K_M and V_{max}; (iii) why we see the particular effect on K_M and V_{max}

10. From the following kinetic data

    a) Using excel or similar graphing program construct a Lineweaver-Burk plot
    b) Determine the V_{max} and K_M for the enzyme without any inhibitor
    c) Determine the V_{max} and K_M in the presence of inhibitors. Determine what type of inhibitor each is

[S] (mM)	v – (uml/ml/sec) No inhibitor	v – (uml/ml/sec) Inhibitor A	v – (uml/ml/sec) Inhibitor B
1	12	4.3	5.5
2	20	8	9
4	29	14	13
8	35	21	16
12	40	26	18

11. Explain how the ping-pong mechanism and sequential mechanism of enzyme reaction works, and how we can distinguish between them using kinetic experiments.